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Behavioral and Neurochemical Effects of Prenatal Halothane

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Permanent neurobehavioral toxicological effects have been theorized to occur at the lowest doses of a toxic agent if exposure occurs during early development compared to exposure during adulthood. Data are reviewed showing that exposure to 10 ppm of halothane from conception to day 60 of life post-partum led to adult rats (≥ 135 days of age) which were hyperalgesic to electric footshock and which committed 30% more errors learning a light-dark discrimination to escape footshock, or learning the shortest path to a food reward in a maze. Exposure only during adulthood to 10 ppm of halothane (from day 60 of life onwards) had no effects. To determine prenatal periods sensitive to halothane, rats were exposed to 12,500 ppm of halothane (with 35% oxygen) on day 3, 10, or 17 of gestation. As adults (≥ 75 days of age) day 3- and day 10-exposed rats, but not day 17-exposed rats, were hyperalgesic and committed 40% more errors in learning a visual discrimination to escape footshock. Food and water consumption, body weight, and running wheel activity were unaffected. Finally, adult rats exposed to 10, 50, or 100 ppm of halothane from conception to day 28 postpartum had 15% less 5-hydroxyindoleacetic acid in brain, but normal 5-hydroxytryptophan, noradrenalin, and dopamine. The possibility is discussed that the hyperalgesia noted above results from a permanently reduced turnover of brain serotonin produced by halothane present in brain at days 10-15 of gestation.

The present paper presents data primarily on the behavioral toxicology resulting from exposure of rats to halothane. Physiopathological data collected in this same research program are presented later in this conference by Chang (*1*).

Since interest in behavioral toxicology is rather recent, the following general comments are in order. The research reported below illustrates some of these comments.

The most useful roles for behavioral measures as a biological endpoint for determining toxicity remain to be empirically defined. Clearly, one role is as a functional test for neurological competence. However, neurological integrity is only one factor in the control of behavior, and one must be alert for behavioral toxic effects which occur through other than neural mechanisms. There are also uncertainties in extrapolating behavioral characteristics from animal models suitable for toxicological study to the human population to which we often want to apply the toxicological data.

Despite these difficulties, the reasons for studying behavioral toxicology are considerable. First, behavioral competence (e.g., in intelligence, drive, emotionality, stability, etc.) may be second only to life itself in importance to the human. Second, the complexity of behavior, and of the brain, suggests that even modest toxic damage might produce alterations. Therefore, neurobehavioral characteristics may be among the most sensitive indicants of toxicity. This sensitivity may be particularly apparent when the neurotoxic effects arise from toxic exposure to the organism during periods of neural development, when the organism is probably most vulnerable to neurotoxic damage. Dobbing (*2*), in particular, has presented a discussion of neurodevelopmental vulnerability. Third, neurotoxic damage inflicted during development, or later, will probably generally prove to be irreversible as pointed out by Mello (*3*). It is therefore important to identify chemicals and dosage which induce neurobehavioral damage before these agents threaten the human condition, since prevention may be the only useful health measure available.

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Experimental and Results

Experiments 1 and 2

As the most vulnerable and sensitive preparation for detecting the neurobehavioral toxicity of halothane, we chose to expose subjects *in utero* and during perinatal life, and to compare the effects on this preparation with effects on adults exposd to halothane. Exposure conditions were chosen to approximate those of chronic exposure of surgical personnel in an operating theater, namely 10 ppm of halothane in ambient air delivered for 8 hr/day for 5 days/week. The subjects were Sprague-Dawley rats, which were dosed (D) or undosed (U) with halothane in early life (i.e., from conception to day 60 of life postpartum) or in later life (from day 60 of life postpartum onwards), according to the following 2 × 2 factorial design. The control rats (condition UU) were not exposed to halothane either in early life or in later life. Other rats were exposed to halothane in early life but not in later life (condition DU), or were not exposed to halothane in early life, but were exposed to halothane in later life (condition UD), or were exposed to halothane in both early life and later life (condition DD).

Although not appreciated at the time, the behavioral tests described below were performed on the third successive generation of rats derived from the above exposure conditions. That is, group DU consisted of rats exposed to the DU condition, whose parents and grandparents had also been exposed to the DU condition, group DD consisted of rats exposed to the DD condition whose parents and grandparents had also been exposed to the DD condition, etc. There is therefore the possibility of some accumulation of halothane effects over generations in the rats finally subjected to behavioral tests.

In experiment 1 (Fig. 1), offspring at either 135 days of age (half the group) or at 150 days of age were trained on a light-dark discrimination in an automated Y-maze to escape an electric footshock (aversive conditioning). In experiment 2 (Fig. 1) offspring at 140-145 days of age were trained to find a path through a variant of the Hebb-Williams maze to reach a goal box containing a food reinforcement (appetitive conditioning). Animal entries into arms of the mazes not leading to the appropriate goal were defined as errors. The complete methodological details have been described previously (*4*, *5*).

As earlier reported for both learning tasks (Fig. 1), the rats exposed to halothane during early life (conditions DU and DD) averaged about 30% more trials on which errors occurred before achieving a learning criterion of 90% error-free trials, compared

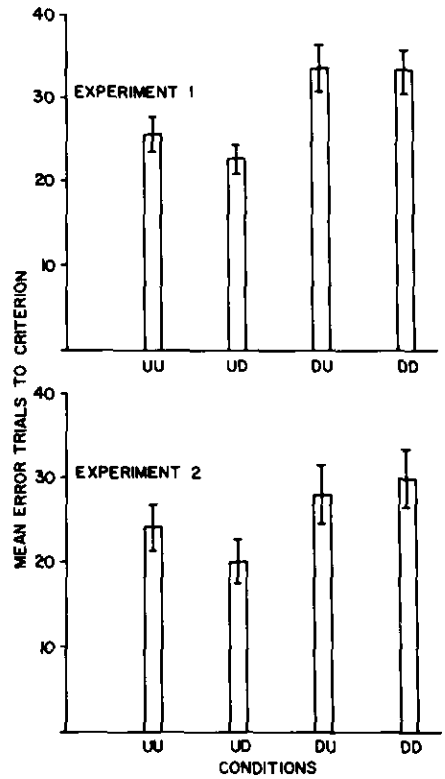

FIGURE 1. Maze learning of rats exposed chronically to 10 ppm of halothane: (top) mean error trials to criterion ± SE for rats of exp. 1 learning a shock escape, light-dark discrimination in a Y-maze; (bottom) mean error trials to criterion ± SE for rats of exp. 2 learning to find the optimum path through a multiple choice point, Hebb-Williams type of maze to obtain food reward. Group designations (UU, UD, DU and DD) are defined in the text.

to controls (condition UU; statistically significant by analysis of variance at $\alpha < 0.05$ in both experiments). Conversely, rats exposed to halothane only during adulthood (condition UD) did not differ significantly from controls in either experiment. Both of the DU groups exhibited retarded learning even though their 83 day period of halothane treatment was terminated at day 60 of age, and at least 75 more days elapsed without halothane exposure before the beginning of behavioral testing. On the other hand, no learning deficits were seen in rats exposed to halothane only as adults (group UD), despite halothane exposure during the 75–90 days immediately prior to behavioral testing, and the continuation of halothane exposure during the days on which behavioral testing was done.

These data strongly suggested that the rats were more sensitive to halothane toxicity if exposed early in life, and that the behavioral effects persisted long after the termination of halothane exposure and might be irreversible.

Experiment 3

Subsequently, rats from the above exposure conditions were tested for their startle-type of responsivity to unsignaled electric footshock at 10–11 months of age (*4*). The rats were placed in a small box on a grid floor and brief electric footshocks were presented once every 15 sec in ascending and descending order of shock intensity (the classical method of limits for threshold determinations). Two thresholds were determined, namely the shock intensity at which 50% of the time the rat flinched to the shock and the higher shock intensity at which 50% of the time the rat jumped or jerked to the shock (a flinch was defined as a body movement without withdrawal of any feet from the grid; a jump was defined as an abrupt withdrawal of one or more feet from the grid).

This test, and the definitions of flinch and jump thresholds, was first described by Evans (*6*) and has been frequently utilized since. The test can obviously serve as a measure of analgesia or hyperalgesia and seems potentially appropriate in assessing possible behavioral consequences of exposure to an anesthetic.

The two groups exposed to halothane in early life (DU and DD) had respective flinch thresholds of 0.33 and 0.46 mA and respective jump thresholds of 0.84 and 1.03 mA. The control (UU) and adult exposed (UD) groups had respective flinch thresholds of 0.62 and 0.58 mA and respective jump thresholds of 1.28 and 1.17 mA. Both the flinch and the jump thresholds of the two early exposed groups combined were significantly lower than the corresponding thresholds of the control and adult exposed groups combined (flinch $F = 4.11$, df = 1,46, $p < 0.05$; jump $F = 5.08$, df = 1,46, $p < 0.05$). That is, the DU and DD groups were hyperalgesic. It is noteworthy that the DU group had been free of halothane exposure for 8-9 months prior to this behavioral test, indicating a permanent behavioral alteration induced by the halothane exposure during development. On the other hand, the UD group had no alteration in either flinch or jump thresholds despite 9 continuous months of exposure at 10 ppm of halothane, 8 hr/day, 5 days/week, prior to and during the flinch-jump test. That is, when exposed only as adults (60 days of age or older), rats appeared resistant to this level of halothane dosage.

Experiment 4

The above data suggested a possible behavioral teratogenic effect of halothane; that is, an effect caused by damage from halothane or its metabolites on developing systems, perhaps neurological, in the rat. There are two developmental periods at which one generally might expect increased vulnerability of neural tissue to toxic exposures. The first of these is the period of organogenesis, peaking for brain at about day 10 of gestation in the rat. Wilson (*7*) has reviewed data showing this to be the earliest and most sensitive period for morphological teratogenicity. The second potentially vulnerable period is the period of most rapid rate of brain growth, which occurs at about day 10-12 postpartum in the rat (*8*).

Dobbing (*2*) has stated the hypothesis and reviewed data for increased neurological vulnerability during periods of greatest rate of brain growth.

The present experiment tested the vulnerability during the first of these periods through prenatal exposure of rats to halothane. A preliminary description of some of these data has previously been given (*9*). Gravid Sprague-Dawley rats were exposed to an anesthetic level of halothane on either day 3, or on day 10, or on day 17 of pregnancy. Exposure conditions consisted of 15,000 ppm of halothane for 5 min to induce anesthesia, followed by 12,500 ppm of halothane for the balance of a 2-hr exposure duration, which maintained anesthesia. The halothane was added to a mixture of 35% oxygen in nitrogen, in an attempt to avoid anoxia. Blood gas measurements (pO_2 and pCO_2) from adult, nonpregnant rats after 2 hr of these exposure conditions indicated no anoxia. However, data of Vannucci (*10*) have indicated neurochemical evidence for anoxia at 10,000 ppm of halothane in enriched oxygen atmosphere despite normal pO_2 in blood.

The offspring from these rats were tested behaviorally beginning at 75 days of age. They were tested first on a very difficult version of visual discrimination in the Y-maze, shock-escape task. The present version of the test required the rats to select the goal arm with a light which flashed on and off at 2/sec versus a goal arm with a steady light of the same intensity. As shown in Figure 2, the control rats (unexposed to halothane) and the offspring exposed on day 17 of gestation, were essentially identical in performance, whereas both the offspring exposed on day 3 of gestation and the offspring exposed on day 10 of gestation took about 40% more error trials to achieve learning criteria of 80% and of 90% correct responding (significantly worse than controls at $p < 0.05$ by t test at both the 80% and the 90% criteria).

At about 110 days of age, these offspring were tested on footshock sensitivity. As in the earlier studies, the rats received ascending and descending series of footshock intensities, one brief footshock every 15 sec. In the present study, however, the

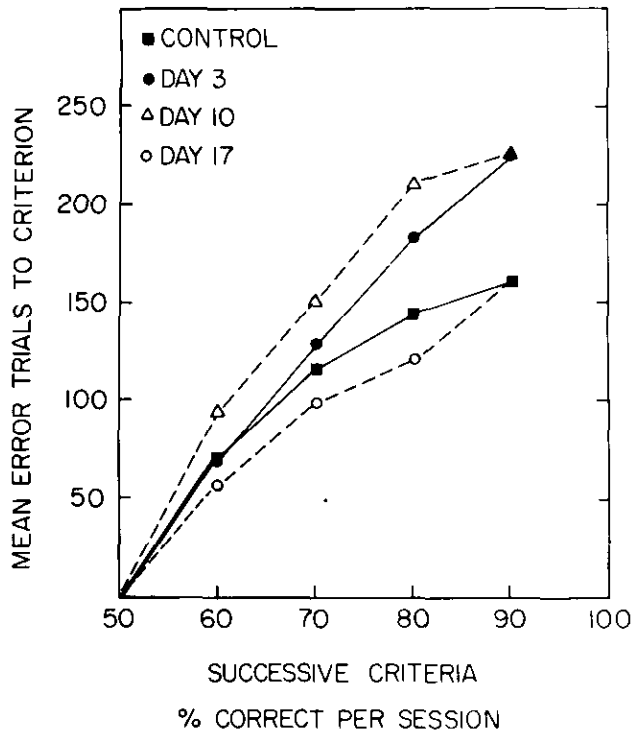

FIGURE 2. Maze learning of rats exposed prenatally for a single, 2-hr period to 12,500 ppm of halothane in 35% oxygen and 65% nitrogen: (■) never-exposed (controls); (●) exposure on day 3; (Δ) exposure on day 10; (○) exposure on day 17 of gestation. Mean error trials to criterion are shown for successively stricter criteria. The rats began by definition of 50% correct, since the single unit Y-maze presented a two-choice situation on each trial.

grid cage holding the rat was suspended on rubber mounts and coupled to a telephone diaphragm. In this way, cage movements produced by body movements of the rat (flinching and jumping) were converted to electrical signals, amplified, and used to drive an analog recording pen. The apparatus was similar to that described by Hoffman and colleagues (*11*). The resultant maximal pen deflection (in millimeters) were averaged for each shock intensity, and the data are shown in Figure 3. On this scale, pen deflections of about 5–40 mm were accompanied by observable flinches, and pen deflections of about 40 mm or more were accompanied by jumps.

As with the Y-maze task, both the control rats and the rats exposed to halothane on day 17 of gestation had similar response amplitudes at each shock intensity. The rats exposed to halothane on day 3 of gestation and those exposed to halothane on day 10 of gestation were hyperalgesic, exhibiting significantly greater response amplitudes by *F*-test ($p < 0.05$) compared to controls.

The behavioral alterations on both the Y-maze task and the footshock sensitivity task were similar

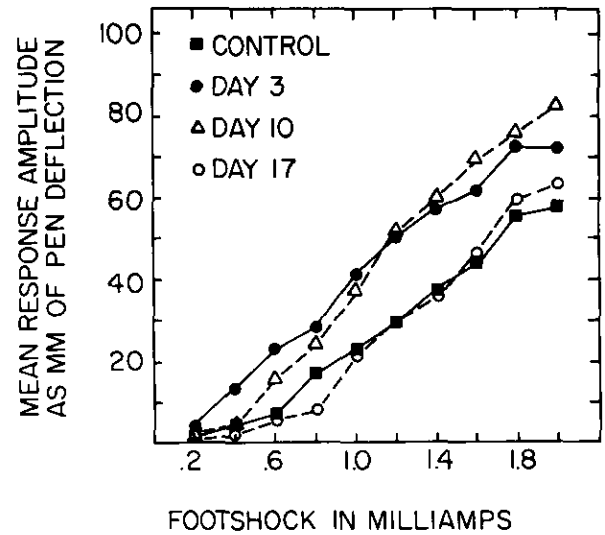

FIGURE 3. Footshock responsivity of rats exposed prenatally to halothane. Symbols as for Fig. 2. The mean response amplitude is shown versus the intensity of unsignalled footshock which evoked the responses.

to those seen in the earlier studies. The vulnerability of rats exposed to halothane on day 10 of gestation was expected, since day 10 represents the period of organogenesis at which teratogenic effects are common. However, the rats exposed to halothane on day 3 of gestation should not have been altered by the exposure, if the halothane effects were limited in time to the preorganogenesis stage. Since halothane is highly retained in lipid, and can be retained in the mammalian body for several days after exposure, we postulate that the gravid rats exposed on day 3 of pregnancy still retained enough halothane on day 10 of pregnancy to produce teratogenic effects in their offspring. If this postulate is correct, then it would also rule out the possibility, at least in the day 3 offspring, that the effects were due to anoxia accompanying halothane anesthesia.

Experiment 5

A number of studies have shown that lowered brain serotonin is accompanied by hyperalgesia as demonstrated by increased responsivity to electric footshock. This is true whether the brain serotonin is depleted by brain lesions or by dosage with *p*-chlorophenylalanine (*12*) or by the feeding of diets low in tryptophan (*13*). In these studies, footshock sensitivities were normalized by restoring brain serotonin levels to normal, either by dosage with 5-hydroxytryptophan (*14*) or by restoring tryptophan to the diet (*13*). This relationship between brain serotonin and footshock responsivity has suggested reason in the present case to investigate

serotonergic function in halothane-treated rats.

Two studies have already reported on brain serotonergic measures in rats treated acutely with halothane (*15, 16*), but not on rats treated chronically or in early life with halothane. In rats recently available from chronic exposures at 0, 10, 50, or 100 ppm of halothane in air, from conception to day 28 of life postpartum, we have measured four brain amines in telencephalon and in diencephalon-midbrain. There were no differences noted in the concentrations of noradrenaline, dopamine, or serotonin in either brain part; however, concentrations of 5-hydroxyindoleacetic acid were 15–20% lower, compared to controls, for all three halothane exposure levels in diencephalon-midbrain (838, 658, 683, and 628 ng/g brain wet weight, respectively; $F = 2.94$, df = 3,24, $\alpha \cong 0.06$) and in telencephalon (558, 515, 536, 480 ng/g brain wet weight, respectively; $F = 3.02$, df = 3,24, $\alpha < 0.05$). These data are consistent with a possible slower turnover of brain serotonin in rats treated in early life with halothane, but more extensive data will be needed to confirm this explanation.

Discussion

The above studies have demonstrated long-term effects on behavior in adult rats resulting from halothane exposure limited to the period of gestation and early life. The effects noted have included deficits in both appetitive and aversive learning tasks, as well as hyperalgesia to mild electric footshock. Other behaviors and variables not discussed here have so far proven to be normal in these same halothane-exposed rats, including body weight, food and water consumption, and running wheel activity. None of these behavioral effects have been seen in rats exposed to the same low doses of halothane only as adults. These data demonstrate an example of behavioral toxicity and its vulnerability and sensitivity to the toxic agent when exposure occurs to the fetus as compared to exposure of the adult.

The mechanisms for these effects have not been established. However, with regard to the permanent hyperalgesic effects of early halothane exposure, the above data are consistent with the tentative hypothesis of serotonergic damage in brain as a mediating factor. Serotonergic neurons have been reported to differentiate in rat brain between days 11-15 of gestation (*16*). If halothane disturbance of this development is the correct explanation of later hyperalgesia, then rats exposed to halothane on day 10 of gestation should display the hyperalgesia, whereas rats exposed to halothane after day 15 (e.g., on day 17) should not display any hyperalgesia. This, of course, is what the above data show.

It must be admitted, however, that the literature has demonstrated an elevated footshock responsivity accompanying reduced concentrations of serotonin, and not accompanying reduced levels of 5-hydroxyindoleacetic acid or reduced serotonin turnover. Although it is plausible (to us) that reduced serotonin turnover may be functionally equivalent in this case to reduced serotonin levels, the case for this hypothesis remains to be definitively established.

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